COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, **Nicholas Kim Hayward** of The Human Genetics Laboratory, Queensland Institute of Medical Research, Herston, QLD 4028, Australia, a research scientist, declare as follows:

1. Professional History.

1.1. I am currently Head of the Human Genetics Laboratory, Queensland Institute of Medical Research (QIMR) I have held this position since 1987. I am also currently an NH&MRC Senior Research Fellow, a Senior Research Fellow at the Queensland Centre for Schizophrenia Research, Wolston Park Hospital, Chairman of the Joint Experimental Oncology Programme of the QIMR, the University of Queensland and the Queensland Cancer Fund, and a conjoint lecturer at the Department of Pathology, University of Queensland.

1.2. Now produced and shown to me marked "**NKH-1**" is a copy of my curriculum vitae, which itemises the publications and presentations of which I have been an author or co-author.

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1.3. I received a Ph.D. in biochemistry from The University of Queensland in 1983. From 1983 to 1990, I was an NH&MRC Research Officer/Senior Research Officer at QIMR, during which time I mapped and identified human genes involved in cancers, such as melanoma, using linkage analysis and positional cloning techniques.

1.4. In 1991, I was awarded an NH&MRC R. D. Wright Fellowship for three years. Following a lecture at QIMR given by Dr J. Shepherd from The Department of Surgery, University of Tasmania, Hobart in about 1989, I became involved in trying to find the gene responsible for multiple endocrine neoplasia type 1 (MEN 1), again using linkage studies and positional cloning techniques. This involved searching the region of human chromosome 11, to which the MEN 1 locus was known to map, for putative genes, identified as open reading frames (ORFs) in DNA clones that we obtained and sequenced. Each candidate ORF that we identified was studied, to determine the possible function of the protein encoded by the ORF. Assigning putative function to each ORF then allowed us to consider whether the ORF was likely to be involved in a suitable physiological pathway that could lead to MEN 1.

1.5. One cDNA clone that we identified early by 1994, although not involved in MEN 1, was determined by us to be highly related to a growth factor known as vascular endothelial growth factor (VEGF). Analysis of the corresponding genomic DNA indicated that the gene encoded two splice variants, one of 167 amino acids and one of 186 amino acids. Careful analysis of these variants revealed that they belonged to the PDGF/VEGF gene family. As of 1994, I was aware of the PDGF/VEGF family of proteins although, until this finding, I was not working with this family.

1.6. After further analysis, I concluded that the gene that I had newly identified was a member of the PDGF/VEGF family of growth factors and was most closely related to VEGF. Consequently I termed the polypeptide encoded by the gene, VEGF-related factor (VRF). This was subsequently changed to VEGF-B in accordance with international nomenclature.

1.7. The results of the cloning work and subsequent sequence analysis that my colleagues and I conducted were published in Grimmond *et al.*, 1996, Genome Research 6: 124-131.

1.8. After isolating human VEGF-B, I went on to clone the corresponding murine orthologue of VEGF-B, including the genomic sequence as well as the cDNA sequence. This work was published in Townson *et al.*, 1996, Biochemical and Biophysical Research Communications 220: 922-928. I also conducted further work to identify and analyse the promoter region of human VEGF-B, which was published in Silins *et al.*, 1997, Biochemical and Biophysical Research Communications 230: 413-418.

1.9. My involvement with VEGF-B is ongoing: I am currently collaborating with Drs Kay and Mould to study the biological role of VEGF-B in mouse development, pathology and neoplasia using, for example, gene knock-out mice defective in VEGF-B. Some of this work has been reported in Bellomo *et al.*, 2000, Circulation Research 86: e29-e35, where it is shown that VEGF-B expression is temporally and spatially regulated, suggesting a role for VEGF-B in ventricular growth.

1.10. In addition to my interest in VEGF-B, during the remainder of the 1990s I continued to work in the field of human cancer genetics, publishing a further 50 scientific papers in this area between 1994 and 1999.

1.11. Thus, I am familiar with the background knowledge, experience and technical abilities of researchers in my field, especially in Australia over the last fifteen years through my research, writing, supervisory responsibilities and referee duties for various international journals. I have been required to develop and maintain a good knowledge of Australian and international scientific literature for a diverse range of scientific fields including molecular biology, biochemistry and cell biology etc. I am very familiar with the field of human genetics and the cloning and analysis of human genes over the last fifteen years. I have published about 100 scientific papers, almost all of which have been concerned with molecular biology and human cancer genetics. In particular, I have direct experience in cloning a member of the PDGF/VEGF family.

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1.12. In the following sections I refer to various scientific publications and patent specifications. Unless otherwise identified, I have not enclosed copies of these documents with this statutory declaration since the patent attorneys representing Human Genome Sciences Inc ("HGS") have informed me that copies of these documents will be filed by separate means.

2. My Instructions

2.1 I am informed by the Patent Attorneys representing HGS that these proceedings concern an opposition by Ludwig Institute for Cancer Research to Australian Patent specification AU-B-696764 (73941/94) by HGS, entitled "Vascular Endothelial Growth Factor 2" ("the patent specification") which has an earliest date of filing of 8 March 1994 ("March 1994"). I have been asked to provide my comments and opinions on the patent specification for use in these proceedings. I have also been asked to provide my comments and opinions as to what the patent specification would provide to one of ordinary skill in the field of molecular biology as of March 1994. My opinions concerning the content (information) in the patent specification are contained in this statutory declaration.

2.2 When I first met the Patent Attorneys for HGS I was provided with a copy of a document entitled "*Guidelines for Expert Witnesses in Proceedings in the Federal Court of Australia*."

2.3 The Patent Attorneys for HGS have provided me with copies of numerous documents. Now produced and shown to me marked "**NKH-2**" is a list of those documents. I have been asked to review these documents and to provide my comments thereon. I do not propose to address each and every paragraph in the statutory declarations that I have reviewed. This decision of mine should not be taken as an admission on my part of acceptance of any text that I do not comment on.

3. Specific comments concerning the patent specification

3.1 In the following paragraphs I comment on the information contained in the patent specification so far as it is relevant to the issues that have been broadly addressed by one or more of Ludwig Institute for

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Cancer Research's expert witnesses. In the next section of this statutory declaration I will provide specific responses to some of the salient paragraphs from Associate Professor Rogers' and Dr Alitalo's statutory declarations.

The meaning of the term "VEGF-2"

3.2 The patent specification provides the nucleotide sequence and amino acid sequence of a 350 amino acid polypeptide, the sequences being shown in Figure 1. This polypeptide is designated VEGF-2. In addition, the cDNA clone that was sequenced to obtain the nucleotide and amino acid sequences shown in Figure 1 has been deposited and in my opinion could readily be sequenced by a skilled person to obtain VEGF-2 sequence information. To be concise, I will confine my comments essentially to polypeptides since the polynucleotide claims are generally limited to sequences that encode VEGF-2 polypeptides and so essentially the same comments apply.

3.3 In biological terms, a protein is governed by its primary amino acid sequence, which determines the structure and function of the protein. However, a protein may exist in a variety of forms that do not have exactly the same primary amino acid sequence but are nonetheless recognisable from the primary amino acid sequence as being the same protein. For example, there may be allelic variants that differ between individuals by a few amino acids. In addition, there may be equivalent proteins in other organisms that have a substantial number of amino acid differences but are nonetheless recognisable as being essentially the same protein (known as orthologues) and not a different protein.

3.4 Consequently, the term VEGF-2 conveys to me the idea of not just the sequences presented in the patent specification, but a family of related sequences. Once a protein has been identified any given sequence can be tested to determine whether any amino acid changes affect the structural and or functional characteristics of that protein.

3.5 Some examples of fragments described in the patent specification include the mature protein lacking the proprotein portion (page 6 third paragraph) and the protein exemplified in Example 2, which is

missing 36 amino acids from its carboxy end. Other fragments could have been produced by 1994 with routine ease using the information in the patent specification.

3.6 The patent specification states that VEGF-2 is involved in angiogenesis (pages 16-17), to promote endothelialisation (page 4 third paragraph and page 17 fourth full paragraph) or tumour angiogenesis and/or tumour neovascularization (page 17 last full paragraph to page 18), as a wound healing agent (page 16 last paragraph that extends to page 17), to treat heart attacks (myocardial infarctions) and cell death due to the loss of blood (ischaemia) (page 17 third full paragraph), and for *in vitro* culturing of vascular endothelial cells (page 18 first paragraph). I therefore consider active fragments to include fragments of VEGF-2 that might for example have one or more of these activities. Alternatively they may have other angiogenic activities that could have been tested using any of the then routinely available assays. Suitable assays were well known by 1994 for determining angiogenic activities including the above activities. For example, *in vitro* three dimensional gel assays could have been used to measure the angiogenic properties of VEGF-2, as reviewed and described in Cockerill, *et al.*, 1994, In: International Reviews of Cytology. A Survey of Cell Biology 159: 113-160 and cell proliferation assays could have been used to measure the mitogenic properties of VEGF-2, as described in Maglione *et al.*, 1991, Proceedings of the National Academy of Sciences (USA) 88: 9267-9271.

3.7 In summary, I consider the phrase "a VEGF-2 polypeptide having the deduced amino acid sequence of Figure 1 or an active fragment, analogue or derivative of said polypeptide" to mean not only the actual human sequence given in Figure 1 but also similar sequences. These sequences may be cloned from other organisms and variants that occur naturally, in the case of allelic variants, or have been produced by mutagenesis procedures (non-naturally occurring variants) as well as chemically modified derivatives and fragments of the above of a suitable size such that they have VEGF 2 biological function or activity, such as *in vivo*, *in vitro* or immunological activity.

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3.8 However, I do not believe, as is asserted by Associate Professor Rogers, that the phrase "fragments, analogues or derivatives" encompasses PDGF, VEGF and PIGF polypeptides or polynucleotides. Specifically, I do not consider PDGF, VEGF or PIGF to be analogues or derivatives of VEGF-2, as each of these proteins are distinct, with each having their own unique amino acid sequence as shown in Figure 2. Further, I do not believe that this view would be shared by a skilled person. The patent specification specifically distinguishes PDGF, VEGF and PIGF, providing a sequence comparison between these three prior art proteins and VEGF-2 and giving their percentage homology to one another (see page 5 last full paragraph and Figure 2 of the specification). In particular I note that there is not sufficient sequence identity between VEGF-2 and PDGF, VEGF and PIGF (as is clearly demonstrated in Figure 2) for these molecules to be considered derivatives of VEGF-2. This provides me with further confirmation that the claims should not be taken to include these proteins.

Polynucleotides - hybridisation

3.9 A number of the claims of the HGS application refer to nucleotide sequences that hybridise to the nucleotide sequence shown in the sequence listing (SEQ ID No. 1) or the cDNA deposited in a specific ATCC deposit. When I read the word "hybridise' in those claims I understand it to mean that the hybridisation reaction should be conducted under suitably stringent conditions such that only VEGF-2 polynucleotide sequences would bind either nucleotide sequence shown in the sequence listing (SEQ ID No. 1) or the cDNA deposited in the ATCC deposit identified in the patent specification or fragments thereof.

3.10 A precise match between the sequence of one DNA and the sequence of another complementary DNA is not necessarily required for hybridization to occur and it is possible to vary the exactness of the match required between a DNA and a complementary sequence by changing the conditions under which annealing takes place. Specifically, the more stringent the hybridization conditions, the greater the match required. The hybridization conditions can be made more stringent by raising the annealing temperature and less

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stringent by lowering the annealing temperature. Similarly, lowering the ionic strength of the solution in which hybridization takes place also increases the hybridization stringency. I and I believe any person of ordinary skill in this field would have been aware of how to vary hybridization conditions to specifically isolate a particular protein such as VEGF-2.

3.11 I note that the patent specification in Example 1 refers to conditions which I would expect are sufficiently stringent to prevent cross-hybridisation between polynucleotides that encode VEGF-2 and other polynucleotides. Here, the patent specification teaches two different hybridisation wash conditions: (1) 60 degrees C with 0.5XSCC and 0.1% SDS or (2) 65 degrees C at 0.2xSSSC. Either of these conditions is sufficiently stringent enough to exclude cross hybridisation with unrelated polynucleotides, particularly, VEGF, PIGF, or PDGF. I also note that page 6 lines 2 to 4 in the patent specification states that no nucleotide sequence homology was detected between VEGF-2, VEGF and both forms of PDGF. The low level of identity between VEGF-2 polynucleotides and other VEGF polynucleotides suggests to me that a researcher would actually get good specificity in hybridisation reactions. In particular, given the low level of homology between VEGF-2 polynucleotides and other PDGF/VEGF polynucleotides it is unlikely that cross-hybridisation would occur between VEGF/PDGF polynucleotides and VEGF-2 polynucleotides at these hybridisation conditions.

3.12 I and I believe any other researcher in the molecular biology field would have no difficulty designing suitable hybridisation conditions to specifically isolate VEGF-2 polynucleotides. I do not read the claims that refer to hybridisation to VEGF-2 in the claims to encompass non-VEGF-2 molecules.

Production of functional VEGF-2.

3.13 The patent specification discloses 350 amino acids of the VEGF-2 sequence whereas it has subsequently been determined that VEGF-2 has 419 amino acids. The missing amino acid sequence is now known to contain the signal sequence that directs secretion of VEGF-2 from the cell.

3.14 The nucleotide sequence shown in Figure 1 actually provides the nucleotide sequence of a further 70 nucleotides of the full VEGF-2 sequence upstream of the putative start codon which encode another 23 amino acids (EATAYASKDLEEQLRSVSSVDEL) in the complete sequence. This means that the nucleotide sequence that encodes 373 amino acids, is given in the HGS application, not 350 amino acids, and therefore only 46 amino acids, not 69 amino acids are missing.

3.15 Since members of the PDGF/VEGF family are growth factors, I would have expected as at March 1994 that any new members would, like VEGF and PDGF, be secreted. In general, most secreted proteins comprise an N-terminal hydrophobic sequence that causes targeting to the endoplasmic reticulum and subsequent secretion (a signal sequence).

3.16 The best way to confirm that a signal sequence functions as such in a biological system is to express the polypeptide containing the signal sequence in a suitable host cell. In the case of VEGF-2 and related molecules this would be a eukaryotic cell.

3.17 The patent specification speculates that the 350 amino acid sequence may contain a signal sequence. If the 350 amino acid VEGF-2 sequence disclosed in the patent specification were expressed in a eukaryotic cell and no secretion observed, I would have looked for reasons why the protein was not secreted in the experimental system. In this respect I was aware by 1994 that it was not uncommon for proteins that were normally secreted to fail to be secreted in an experimental system.

3.18 There may be a number of reasons why a recombinant protein may fail to be secreted. For example, the signal sequence may be silent or inefficient in a particular context. Alternatively, the signal sequence may be incomplete or missing.

3.19 I do not consider the lack of a disclosure of the N-terminal amino acids containing the signal sequence to be a critical omission from the patent specification. The specification describes utilising a heterologous signal sequence capable of directing secretion of the translated protein (see, the patent specification at page 14, lines 6-

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23). Thus, there is a disclosure in the patent specification to fuse the sequence provided to a signal sequence that directs secretion of the protein. By 1994 a number of signal sequences were known and could have been used to direct secretion of a heterologous protein. Even if the disclosed VEGF-2 sequence contained an atypical signal sequence, I would still have linked the sequence disclosed in the patent specification to a strong signal sequence to ensure efficient secretion of the protein. Such research would have been standard practice for me and I believe my colleagues by 1994.

3.20 Consequently, I believe that if the 350 amino acid sequence was expressed but not secreted then I and I believe my colleagues would simply add a signal sequence to ensure efficient secretion. I would do so rather than assume that the 350 amino acid VEGF-2 sequence was not a secreted protein because of the striking conservation of the eight cysteine residues in the middle portion of the molecule, the presence of the 14 amino acid PDGF/VEGF signature motif, and the overall homology with VEGF which was known to be a secreted growth factor.

3.21 The addition of signal sequences was routine by 1994. Indeed, a number of commercial expression vectors were available with signal sequences already present. All a researcher had to do was to clone their sequence downstream of and in frame with the signal sequence. Examples of suitable vectors that were available by 1994 include the Baculovirus Transfer Vectors: pMbac and pPbac described in the 1994 Stratagene Cloning Systems Catalogue on page 45. These vectors have sequences encoding the secretory signal peptides from human placental alkaline phosphatase or mellitin upstream of their cloning sites. Another example is the prokaryotic vector pEZZ 18 (Pharmacia LKB Biotechnology: Molecular and Cell Biology Catalogue 1992, page 5) which contains a protein A signal sequence to allow secretion of the protein into culture medium.

3.22 I am aware that VEGF-2 is proteolytically processed upon secretion from cells *in vivo* to form the naturally occurring ligand for the Flt-4 and the KDR/Flk-1 receptors. I would expect a fusion of the 350 amino acid sequence of VEGF-2 to a signal sequence to be proteolytically processed to produce functional VEGF-2.

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3.23 Thus I consider that the patent specification provides all the necessary information to routinely obtain fully active processed VEGF-2.

3.24 I have been asked to review the first priority document for Helsinki/Ludwig patent specification 711578 (66169/96). I have done this and note that the document shows that the inventors of that patent application also (i) obtained a partial clone encoding a polypeptide of 350 amino acids, (ii) predicted the start methionine to be in exactly the same place as in the HGS patent specification, and (iii) predicted a signal sequence at the start of the 350 amino acid sequence. Further, I am aware that Dr. Alitalo reported these results in Joukov *et al.*, 1996, EMBO Journal 15: 290-298. He subsequently corrected this statement in Joukov *et al.*, 1996, EMBO Journal 15: 1751

3.25 Once the nucleotide sequence of the majority of VEGF-2 had been made available, a skilled person would realise from routine experimentation that the 5' end of the gene was missing from the clone and seek to obtain the additional sequence, using the available sequence to do so. Once it had been established that the clone was a partial clone, it would, be a routine matter to carry out further screening to obtain the full length sequence. Regardless, there is sufficient information in the 350 amino acid form to allow for the expression of a biologically active form of VEGF-2 that is correctly processed.

3.26 I consider the information provided in the patent specification sufficient to allow myself and any other skilled person to practice the invention in relation to expressing functional VEGF-2. In addition, it would have been by 1994 a routine matter firstly to recognise that there was some sequence missing from the VEGF-2 clone and secondly to obtain the additional sequence encoding the N-terminal 46 amino acids of VEGF-2.

Biological activities of VEGF-2 and uses

3.27 The patent specification provides numerous references to examples of properties and activities of VEGF-2 (see, for example, pages 4 and 16 to 17). At the very least, the actual name given to the protein

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would indicate to me that the protein is a vascular endothelial growth factor and therefore would be expected to act as a mitogen towards vascular endothelial cells. It would also direct me to possible means for testing for biological function or activity of that molecule.

3.28 The patent specification discloses that VEGF-2 is involved in angiogenesis (pages 16-17), as a wound healing agent (page 4 third paragraph and page 16 last paragraph that extends onto page 17), to promote endothelialisation (page 4 third paragraph and page 17 fourth paragraph), to treat myocardial infarctions and ischaemia (page 17 third full paragraph) and for *in vitro* culturing of vascular endothelial cells (page 18 first paragraph). The specification also discloses at page 17 last full paragraph extending to page 18 that VEGF-2 is involved in tumour angiogenesis.

3.29 I do not consider the various properties and uses described in the patent specification as exhaustive. The patent specification teaches that VEGF-2 is a member of the PDGF/VEGF family of growth factors. On reading this by 1994 a skilled person would have, as a matter of routine, subject VEGF-2 to a variety of tests and experimental procedures already known in the art for VEGF. For example, the VEGF-2 molecules could be tested using endothelial cell proliferation assays, angiogenesis assays and wound healing assays. All these types of assays were routine by 1994. Given the identification of VEGF-2 as a member of the PDGF/VEGF family of growth factors, I and I believe my colleagues would be looking to see if VEGF-2 actually functioned as a growth factor, which of course it does.

3.30 In addition, page 4 lines 12 to 14 of the patent specification states that the VEGF-2 polypeptides of the invention may be used to isolate receptors of VEGF-2. At page 24 fifth paragraph to page 25 first paragraph the patent specification discloses that VEGF-2 binds to tyrosine kinase receptors on the surface of target cells to activate endothelial cell growth.

3.31 In 1990, a receptor for VEGF was identified, the Flt1 receptor (also known as VEGFR-1) (Shibuya *et al.*, 1990, Oncogene 5: 519-524).

3.32 A further VEGF receptor, the Flk-1/KDR receptor (also known as VEGFR-2) was reported in 1991 (Terman *et al.*, 1992, Biochemical

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and Biophysical Research Communications 187: 1579-1586 and Terman *et al.*, 1991, Oncogene 6: 1677-1683). Both receptors were characterised as members of the receptor tyrosine kinase superfamily. Binding of VEGF to the receptor results in receptor phosphorylation.

3.33 In 1992, (Aprelikova *et al.*, 1992, Cancer Research 52: 746-748) a further orphan receptor, termed "Flt4", was described as a class III receptor tyrosine kinase with significant homology to the Flt1 receptor. The Flt4, Flk1 and Flk1/KDR receptors were referred to as the FLT gene family, tyrosine kinase receptors which bind VEGF molecules, in Kaipainen *et al.*, 1993, Journal of Experimental Medicine 178: 2077-2088 (last sentence of abstract on page 2077).

3.34 Given (i) that the patent specification states that the VEGF-2 protein may be used to isolate receptors for VEGF-2, and (ii) that the patent specification states that VEGF-2 may bind to tyrosine kinase receptors and (iii) the availability of three members of a family of receptors, one of which had no known ligand while the other two were known to bind VEGF, I would expect a skilled person to consider whether the VEGF-2 protein could bind to any one of Flt1, Flk1/KDR and Flt4.

3.35 Since the patent specification teaches a new member of the PDGF/VEGF family with no known receptor and the prior art teaches an orphan receptor in the same family as the receptor for VEGF with no known ligand, I and I believe others in my field would consider it a matter of routine to test VEGF-2 for binding to the Flt-4 receptor, the Flt1 receptor and the Flk-1/KDR receptor.

3.36 I consider the patent specification to teach a number of properties of and uses for VEGF-2. Furthermore, I and I believe others of ordinary skill in my field would be able to use the information provided in the patent specification to produce VEGF-2 protein and confirm that VEGF-2 does indeed have the properties discussed above, as well as obtaining further information about VEGF-2, using a variety of routine techniques already in use by researchers by 1994 to study growth factors such as VEGF.

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Uses of fragments of VEGF-2 and VEGF-2 antibodies

3.37 Associate Professor Rogers has commented that fragments of polypeptides have no practical utility whatsoever (see for example paragraph 4.10.2, Associate Professor Rogers' Statutory Declaration). Clearly this is not the case. As asserted in the patent specification, fragments of polypeptides can be used to make antibodies, which are useful both experimentally and therapeutically. In addition, active fragments of polypeptides are often used in therapy as antagonists since they can compete with the full length version but may lack full biological activity. Further, such polypeptide fragments could also be used as agonists that might mimic some of the biological activities of the full-length protein. Although the skilled person would be aware of this in any case, I note that the specification discloses the following:

3.37.1 Page 22 last full paragraph that extends to page 23 teaches that fragments may be used to make antibodies to the polypeptide [VEGF-2]. Pages 22-23 of the patent specification describe suitable techniques for producing antibodies that bind to VEGF-2

3.37.2 Page 23 last paragraph to page 24 second paragraph teaches that the antibodies may be used to block the activity of VEGF-2, in a similar manner as has been demonstrated previously for VEGF.

3.37.3 Page 24 first paragraph teaches that VEGF-2 antibodies may be used to measure elevated levels of VEGF-2 in individuals. It is in fact common for cancerous conditions to be associated with increased levels of growth factors.

3.37.4 Further, the patent specification clearly demonstrates that VEGF-2 is expressed at higher levels in cell lines derived from malignant tumours (page 18 lines 1 to 5).

3.37.5 Page 24 fifth paragraph teaches that truncated versions of VEGF-2 that fail to activate endothelial cell growth may be used as an antagonist.

4. Comments on Associate Professor Rogers' Statutory Declaration

4.1 Associate Professor Rogers has raised a number of points in his statutory declaration. However, many of the points are repetitive and so I have sought to deal with them in a more structured manner. Consequently, a lack of reference to a specific item in Associate Professor Rogers' statutory declaration should not be taken as an admission that I necessarily agree with his comments.

The meaning of VEGF-2

4.2 One issue that Associate Professor Rogers comments on is the meaning of "VEGF-2 fragments, analogues and derivatives". I set out in paragraphs 3.4 to 3.8 my understanding of these terms from my reading of the patent specification. I would reiterate that, by contrast to Associate Professor Rogers' comments in paragraph 2.2 and 2.3 of his statutory declaration, I do not consider VEGF-2 fragments, analogues and derivatives to encompass PDGF, VEGF or PlGF polypeptides or polynucleotides. Specifically, I do not consider PDGF, VEGF or PlGF to be analogues or derivatives of VEGF-2 in the same way that I do not believe that a skilled person would consider VEGF to be a fragment analogue or derivative of PDGF. They are different molecules.

VEGF-2 activity

4.3 Associate Professor Rogers asserts in his declaration that the patent specification has not taught a unique defining activity that can serve to discriminate PDGF/VEGF from VEGF-2. However, I do not see how this is relevant, nor do I agree that it is necessary to do so. Once a substantial portion of the primary amino acid sequence is available, this provides the essential defining characteristic.

4.4 Associate Professor Rogers also makes the general point that he considers the patent specification does not provide a demonstration or guidance as to VEGF-2 biological activity (paragraph 2.3.2, paragraph 4.6). However, I consider that the patent specification provides a variety of information and guidance about the biological activity of VEGF-2 (e.g. on page 4 and pages 16 to 18 of the patent specification). I refer to and repeat my detailed comments in

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paragraphs 3.27 to 3.39 and 3.37. Furthermore, I, and I believe a skilled person when presented with the information in the patent specification, would be able to obtain confirmation of these biological activities using techniques that were routinely available and used by 1994, such as angiogenesis assays and endothelial cell proliferation assays referred to in paragraph 3.29 above.

4.5 Associate Professor Rogers states in paragraph 4.6.1 of his statutory declaration:

> "...that the specification fails to demonstrate any VEGF2 biological activity is especially significant in that the biological activities of VEGF2 (if any) is a concept analogous to utility: if one does not know the activities of VEGF2, one would not know how to use VEGF2 (except as a curiosity for further research)"

4.6 In Example 1 in the patent specification the inventors demonstrate that VEGF-2 is over expressed *in vivo* in a number of malignant breast tumour cell lines. This result is, I believe, indicative of VEGF-2 biological activity. It suggests to me that VEGF-2 plays a role in tumour development possibly by promoting new blood vessel growth similar to VEGF. Additionally, it is apparent to me from reading the patent specification as I believe it would be to others in my field that VEGF-2 is a growth factor that is related to VEGF and is likely to play a role in the regulation of endothelial cell mitogenesis.

4.7 I am unaware of any evidence that suggests that VEGF-2 does not possess the activities identified in the patent specification. Further, by 1994 it would have been routine for a skilled person to confirm that these proposed activities were correct. For example, testing polypeptides for effects on the growth of cells such as endothelial cells (as set forth on page 18 lines 6 to 8 of the specification) was routine by 1994. It was also routine for a skilled person to test for wound healing using a standard punch biopsy.

4.8 I also add that neither I nor any of my colleagues would expect that the initial characterisation of a gene should provide a full description, supported by detailed experimental proof, of every property and function that the encoded protein possesses.

4.9 Further, once the VEGF-2 sequence of the patent specification was identified, I do not believe that an ordinarily skilled person would

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have had any difficulty by 1994 in producing analogues, fragments and derivatives of VEGF-2. The same routine ease would have also applied to the testing of VEGF-2 analogues, fragments and derivatives for VEGF-2 activity.

4.10 In response to paragraph 5.5 (5.5.1) of Associate Professor Rogers' declaration, I consider the process of determining which parts of a polypeptide molecule are required for activity to have been entirely routine by 1994. Suitable strategies would have included deletion mutagenesis or site-directed mutagenesis, which involves deleting or replacing regions of the molecule and determining the effect on protein function. Additionally, the specification highlights the 8 cysteines and the 14 amino acid signature motif thought to be involved in VEGF-2 function.

Hybridising and immunologically cross-reactive polypeptides

4.11 In paragraph 2.4 of his statutory declaration, Associate Professor Rogers' comments that the limitation "hybridising to" does not serve to distinguish the claimed polynucleotides from polynucleotides encoding VEGF, PlGF and PDGF. However, as stated in paragraphs 3.9 to 3.12 above, I consider that it is unlikely that there would be any cross-hybridisation between VEGF-2 polynucleotides and polynucleotides encoding VEGF, PlGF and PDGF under suitable hybridisation conditions such as those provided in Example 1, particularly given the lack of detectable homology between VEGF-2 and other members of the PDGF/EVGF family (page 6 lines 2 to 5 of the specification) at the polynucleotide level.

4.12 Further, in response to Associate Professor Rogers comments in paragraphs 2.7.5 and 4.10.5, I do not consider it likely that a polynucleotide hybridising under these conditions and cross-reactive with an antibody that binds to VEGF-2 would encode anything other than a VEGF-2 molecule or a immunologically reactive fragment of VEGF-2. The dual test provided by claims 16 to 27 and 40 to 50 (when read in conjunction with the specification) is, I believe, actually much more stringent than Associate Professor Rogers has alleged. I am not aware of any polypeptides/polynucleotides that would satisfy the criteria of claims 16 to 18 and 40 to 45 but which are not VEGF-2 or an immunologically reactive fragment of VEGF-2.

4.13 Similar comments apply to Associate Professor Rogers' statements in paragraph 4.10.1 where he draws attention to the lack of an explicit reference in claims 16 to 18 and 40 to 45 to "activity". However, the polypeptides encoded by the polynucleotides referred to in these claims are all immunologically cross-reactive with an antibody that binds to a VEGF-2 polypeptide, and are therefore biologically active fragments. Consequently, I consider these claims to require implicitly the polypeptides to have VEGF-2 activity.

4.14 My comments in paragraphs 3.3 and 3.8 above are also relevant to paragraphs 5.7.2 to 5.7.3 where Associate Professor Rogers has referred to the "enormous scope of these claims". For the reasons set out in paragraphs 3.2 to 3.8, I believe that the claims are limited to VEGF-2 polypeptides or a immunologically reactive fragment of VEGF-2.

4.15 Associate Professor Rogers comments in paragraph 5.7.1 that the language used in claims 16 to 18 and 40 to 45 that refers to "a polypeptide which binds to an antibody capable of binding to VEGF-2" is not found in the specification. However, page 23 lines 5 to 14 refer to polypeptides that are bound by antibodies generated against polypeptides of the invention. Since the polypeptides of the invention are VEGF-2 polypeptides, I interpret this section as corresponding to a disclosure of a polypeptide that is bound by an antibody to VEGF-2.

Production of antibodies

4.16 Associate Professor Rogers also comments in paragraph 4.10.2 that no antibodies are exemplified in the specification. In my opinion, given the disclosure of the VEGF-2 sequence in patent specification, biological scientists, from Ph.D. students to heads of Department, would have had no difficulty by 1994 in obtaining antibodies to VEGF-2 using routine techniques such as immunisation of rabbits with purified VEGF-2 protein. They would not need a demonstration in the patent specification to enable them to do this. Nonetheless, page 23 second and third paragraphs of the patent specification describes suitable techniques for producing antibodies.

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Disclosure of VEGF-2 sequence

4.17 In paragraph 4.4, Associate Professor Rogers comments that the patent specification fails to disclose the complete amino acid and polynucleotide sequence of VEGF-2. In particular, Associate Professor Rogers comments in paragraph 4.11.1.2 that the patent specification fails to teach the N-terminal 69 amino acids of the full length molecule which contains the signal sequence "which is crucial for directing secretion of VEGF2 in cells". I should note that whilst a signal sequence is necessary for secretion, there is no requirement to use the signal sequence that is normally found at the N-terminus of the newly expressed VEGF-2 molecule.

4.18 The thrust of Associate Professor Rogers' arguments appears to me to be that HGS should not be entitled to claim the full length VEGF-2 molecule because only polynucleotides encoding 350 amino acids (or 373 amino acids in actual fact) are disclosed in the patent specification. However, I consider that the information provided in the patent specification would allow the skilled person to obtain the complete sequence without any difficulty, as discussed in 3.13 to 3.26.

4.19 In this regard, I also consider that the information provided in the patent specification would allow the skilled person to obtain VEGF-2 nucleotide sequences from other species (so-called orthologues) by routine techniques. Consequently, I disagree with Associate Professor Rogers' comments in paragraph 5.6 that the specification provides no basis for non-human forms of VEGF-2.

4.20 Associate Professor Rogers also appears to consider that the specification does not teach a VEGF-2 molecule that possesses biological activity because the sequence of the N-terminal 69 amino acids found in the full length molecule is not provided in the patent specification (see for example paragraph 7.6). I have set out my comments at length in paragraphs 3.13 to 3.26 as to why I believe that the specification teaches the skilled person how to obtain biologically active VEGF-2. In particular, I believe that faced with any difficulties in obtaining secretion of the 350 amino acid VEGF-2 polypeptide disclosed in the specification, I and I believe others of ordinary skill in the PDGF/VEGF field, would simply add/substitute a

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signal sequence and the resulting protein would be properly processed by a suitable host cell to produce active VEGF-2. As demonstrated by Dr Alitalo's own work, fragments smaller than the 350 amino acid sequence, which retain the signature motif and cysteine residues, contain sufficient information to be processed to a biologically active form. (See, U.S. Patent No. 6,130,071, issued October 10, 2000, to Alitalo et al., column 47 line 57 to column 48 line 2)

Examples in the Patent Specification

4.21 Associate Professor Rogers has made some comments in paragraph 4.13 on a number of apparent errors.

4.22 In paragraph 4.13.1. Associate Professor Rogers comments that the size of the mRNA referred to in Example 1 (1.6 kD) is inconsistent with subsequent Northern hybridisation studies. The 1.6 kD actually refers to a Northern blot shown as Figure 4 where a number of breast cancer cell lines were tested. Two strong bands are seen in lanes 4 and 6 of the autoradiograph shown in Figure 4. These two lanes correspond to total RNA from two breast tumour cell lines. The size markers on the right hand side appear to me to correspond to 18S and 28S ribosomal RNA, which have molecular weights of about 2.2 and 4.5 kb, respectively. Thus the strong bands in lanes 4 and 6 are clearly less than 2.2 kb in size and 1.6 kb would not be an unreasonable interpretation. (As a minor point the reference to "kD' in the specification and not "kb" is obviously an error and one I would expect to be recognised by one of ordinary skill. Such a person would appreciate that "kb" was the intended term since the size of RNA on a Northern blot is measured in kilobases not kiloDaltons).

4.23 Interestingly, a corresponding band is not seen in the lane corresponding to normal breast cells or in most of the other breast tumour cell lines. Consequently, it appears likely to me that the results shown in Figure 4 show that a 1.6 kb RNA that hybridises to a VEGF-2 probe is highly over-expressed in two breast tumour cell lines.

4.24 Figure 5, by contrast, shows the results obtained with a range of normal cells from different tissues i.e. Figures 4 and 5 show the

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results of different experiments. The two bands seen in lanes 1 to 10 are indicated on Figure 5 to be 2.2 kb and 1.3 kb in size. However, the 1.3 kb band has a greater molecular mass than the 1.4 kb molecular weight marker on the left hand side of the Northern blot. I would judge the two bands to be about 2.4/2.3 kb and 1.6 to 1.8 kb. Thus at least the major band is reasonably consistent with subsequent results.

4.25 Although there are two minor errors with respect to "kD' instead of "kb" and a mislabelled band in Figure 5, I had no difficulty in understanding the results presented.

4.26 In paragraph 4.13.3 of his statutory declaration, Associate Professor Rogers has criticised the expression studies conducted in Example 2 of the patent specification. Specifically, Associate Professor Rogers has commented that the lack of a sequence for the F5 primer means that it is not clear how the third primer pair can produce a PCR product that encodes the full polypeptide of VEGF-2. I disagree. Since the sequence of the complete VEGF-2 cDNA insert is given in Figure 1 of the specification, it would be a simple matter for the skilled person to design a suitable primer that hybridises to a region between the VEGF-2 stop codon and the 3' end of the insert. Indeed, it would be a simple matter for the skilled person to produce a variety of different length PCR products containing all or some of the VEGF-2 cDNA insert with the information provided in Figure 1.

4.27 With regard to the second primer pair that produces a PCR product encoding a polypeptide missing 36 amino acids from the C-terminus of VEGF-2, there does appear to be an error in Example 2. The specification states that the F4 primer is "from bp 1259 to 1239, about 169 bp away from the 3' end of the stop codon and about 266 bp before the last nucleotide of the cDNA." (page 28 lines 22 to 26). When compared with the sequence of Figure 1, this is clearly within the 3' UTR. Consequently, the sequence given for the F4 primer when combined with an M13 reverse primer would give the full VEGF-2 product. It would therefore appear to me that the F4 primer sequence is actually the F5 primer sequence and the F4 primer is missing.

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4.28 Since Example 2 teaches that the second primer pair amplifies a PCR product encoding a polypeptide missing 36 amino acids from the C-terminus of VEGF-2, and the nucleotide sequence of VEGF-2 is given in Figure 1, it would have been possible for me to design by 1994 a suitable primer using the Figure 1 information. I have in fact designed such a primer as an example, using only the information in the specification. The primer sequence is: 5' ACAGCTGCATGTTT GGTGG 3'. Therefore the fact that the primer is missing is not in my opinion a problem.

4.29 While there are some errors in Example 2, it clearly shows that the VEGF-2 cDNA can be expressed *in vitro* to produce a polypeptide product. In any case, with the information provided in the specification, in particular Figure 1, the skilled person could easily carry out similar experiments to those shown in Example 2 without any specific guidance as to the primer sequences etc., and obtain similar results.

5. Comments on Dr Alitalo's Statutory Declaration

5.1 Dr Alitalo describes in his statutory declaration some expression studies using expression plasmids containing a construct encoding amino acids 70 to 419 of full length VEGF-2 (i.e. amino acids 1 to 350 of VEGF-2 as described in the patent specification) or a construct encoding the complete 419 amino acid sequence of VEGF-C.

5.2 The plasmids were transiently transfected into a mammalian cell line (293T cells). Both cell lysates and the culture medium were tested for the presence of newly synthesised VEGF-2/VEGF-C proteins (which are radioactively labelled due to the inclusion of radioactively labelled methionine in the culture medium). The results are shown in Exhibit 3.

5.3 VEGF-2/VEGF-C were partially purified from the cell medium and cell lysates using an immunoprecipitation procedure. This involves using antibodies to bind to the VEGF-2/VEGF-C. However, as discussed in paragraph 6.5 of Dr. Alitalo's statutory declaration, the antibody used to immunoprecipitate VEGF-2(70-419) was a monoclonal antibody that recognises hemagglutinin (HA), a peptide tag which was fused to the C-terminus of VEGF-2. By contrast, the antibody used to

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immunoprecipitate VEGF-C (1-419) was a polyclonal antibody, which recognises residues 31 to 51 of the 350 amino acid VEGF-2 polypeptide.

5.4 In Alitalo's own published work, he has reported the inability to isolate VEGF-C using an antiserum against the C-terminal amino acid residues 372-394 or by using a tag attached to the C-terminus (Joukov et al., 1997, EMBO J. 16: 3898, at 3900). Thus, by Alitalo's own admission an antibody to a tag at the C-terminus of VEGF-2 will not successfully immunoprecipitate the protein.

5.5 HGS scientists have reported the successful isolation of a modified VEGF-2 protein containing an HA tag at its carboxy terminus using a monoclonal anti-HA antibody. (See, HGS Australian Patent No. 714484 and Hu JS et al (1997) FASEB J May;11(6):498-504). These studies were conducted in COS cells, whereas the experiments set forth in Dr. Alitalo's declaration were conducted in 293T cells. The significance of the different cell types used is provided by Dr. Alitalo's own publications (Joukov et al., 1997, EMBO J 16: 3898-3911). This publication describes the proteolytic processing of VEGF-2 when expressed by a number of different cells lines, including COS cells, PC-3 cells, HT 1080 cells, and 293 EBNA cells. The results of this comparison, as reported by Dr. Alitalo was that "[t]he proteolytic processing of the VEGF-C precursor in COS cells was less efficient when compared with other cell types." (See Joukov, at page 3901, second column). Thus, as the VEGF-C precursor is processed less efficiently in COS cells, one would also expect that the cleavage of the HA tag from the carboxy terminus would also be less efficient in COS cells, as compared to 293T cells. Hence, the lack of efficient cleavage of the HA tag from the carboxy terminus of the protein when expressed in COS cells may account for HGS' successful isolation of VEGF-2 protein containing an HA tag at its carboxy terminus.

5.6 It is not clear to me why different antibodies were used, particularly since the VEGF-C antibody would have recognised both VEGF-C and VEGF-2. Importantly, however, the use of two different antibodies introduces three major flaws into the experimental design, which prevent conclusions from being drawn from the data obtained and shown in Exhibit 3.

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5.7 First, even different antibodies that bind to the same polypeptide tend to have different affinities for the region of the polypeptide to which they bind. This means in practical terms that for a given amount of the protein, the amount detected or bound by each antibody often differs. Thus, the results obtained with two different antibodies cannot be compared quantitatively due to the lack of any controls. In this case the antibodies are even against different proteins (VEGF-C and HA). Consequently, it is not possible to make any kind of quantitative comparison between the results for VEGF-C and VEGF-2 shown in Exhibit C since the differing efficiency with which the different antibodies bind to their target polypeptides will affect the proportion of protein that they are able to bind in the culture medium and cell lysates. Furthermore, one antibody is monoclonal and one is polyclonal and so a comparison of the results is even more difficult to make. Also, no indication is given as to the amount of polyclonal antibody used.

5.8 Second, the aim of the experiment is to determine whether VEGF-2(HGS) is processed and secreted by a mammalian cell. However, whereas the antibody used to detect VEGF-C binds an epitope which is present in the fully processed VEGF-C molecule, the antibody used to detect VEGF-2 binds to a heterologous fusion protein tag (HA) located at the C-terminus of the unprocessed molecule. If VEGF-2 is proteolytically processed to form mature VEGF-2/VEGF-C, the HA tag will be cleaved from the mature part of the molecule. The mature VEGF-2 molecule, no longer being linked to the HA tag, would not be immunoprecipitated and would not therefore appear in either the immunoprecipitated cell medium or immunoprecipitated in the cell lysate, having being discarded when the protein A-sepharose is washed. Thus, in the case of VEGF-C expression, the experimental design allows for the detection of both unprocessed and mature VEGF-C whereas in the case of VEGF-2 expression, only the unprocessed form can be detected and not the mature form. Furthermore, Alitalo's own work indicates that an antibody to a C-terminal tag may not be effective in isolating VEGF-C.

5.9 It is not therefore possible to draw any conclusions about the relative efficiency of secretion of VEGF-C and VEGF-2 (70 to 419) from these data - the same antibody should have been used in both cases.

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5.10 Third, no controls have been carried out to determine the transfection efficiency of the plasmids used - making any comparisons even more difficult.

5.11 Dr Alitalo also states in paragraph 7.2 that

> "it is readily apparent from the autoradiogram [Exhibit 3] that the expression level of VEGF-C is much higher than that of VEGF2(HGS)".

5.12 Further, Dr Alitalo states in paragraph 8.3 that

> "the fact that VEGF-C expression observable in cell lysates of VEGF-C transfected cells is much higher than VEGF2(HGS) expression observable in VEGF2(HGS) transfected cells suggests that VEGF2(HGS) is inefficiently translated and/or that the intracellular turnover rate of VEGF2(HGS) is much faster than that of VEGF-C. In other words, the cells may be recognizing VEGF2(HGS) as an aberrant protein and rapidly degrading it".

5.13 Neither of these conclusions are supported by the flawed data shown in Exhibit 3. As discussed in paragraph 5.3 to 5.6 above, it is not possible to draw any conclusions from these data based on quantitative comparisons. The conclusions set out in paragraphs 7.2 and 8.3 are mere speculation that is unsupported by the results obtained.

AND I make this solemn declaration by virtue of the Statutory Declarations Act, 1959 and subject to the penalties provided by that Act for the making of false statements in statutory declarations, conscientiously believing the statements contained in this declaration to be true in every particular.

DATED this day 8th of December 2000.

DECLARED at: Brisbane, Queensland)
BEFORE me: .....)

N. Hayward
.....
Nicholas Kim Hayward

.....
Patent Attorney

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COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

OPPOSITION THERETO BY: Ludwig Institute for Cancer Research Under Section 59 of the Patents Act.

This is **Annexure NKH-1** referred to in my Statutory Declaration made this 8th day of December 2000.

N. Hayward

Nicholas Kim Hayward

WITNESS: _____

Commissioner for Declarations/Solicitor
Patent Attorney/Justice of the Peace

CURRICULUM VITAE

NAME: Nicholas Kim Hayward

WORK ADDRESS: Human Genetics Laboratory
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TERTIARY QUALIFICATIONS:

1981-1983 Doctor of Philosophy, Biochemistry Department, University of Queensland - project titled "Phenacetin metabolites and DNA damage". Conferred 7/7/83.

1980 Master of Science, Qualifying, Biochemistry Dept. University of Queensland (UQ) - passed at the level of first class honours.

1977-1979 Bachelor of Science, University of Queensland - majoring in Biochemistry and Microbiology.

POSITIONS HELD:

Current:

- NH&MRC Senior Research Fellow.
- Head, Human Genetics Laboratory, Queensland Institute of Medical Research (QIMR).
- Chair, Cancer and Cell Biology Division, QIMR
- Chair, Joint Experimental Oncology Programme of the QIMR, the University of Queensland and the Queensland Cancer Fund.
- Conjoint lecturer - Department of Pathology, University of Queensland.
- Senior Research Fellow, Queensland Centre for Schizophrenia Research, Wolston Park Hospital, Wacol, QLD 4076.
- Faculty Representative, QIMR Staff Association
- Faculty Representative, QIMR Scientific Advisory Committee
- Faculty Representative, QIMR Joint Consultative Committee

Previous:

1994-1997 NH&MRC Research Fellow
1991-1993 NH&MRC R. D. Wright Fellow
1983-1990 NH&MRC research officer/senior research officer - QIMR.
1982 Group 1 tutor: Medicine and Pharmacy students - University of Queensland.
1982 Private tutor: Medical students - University of Queensland.
1980-1981 Casual tutor: Science, Dentistry and Pharmacy students - University of Queensland.

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AWARDS:

NH&MRC Senior Research Fellowship 1998-2002.

NH&MRC Research Fellowship 1994-1997.

Queensland Cancer Fund Travel Fellowship 1994.

NH&MRC R. D. Wright Fellowship 1991-1993.

Beckman-QIMR Young Scientist Award 1991.

Queensland Cancer Fund Travel Fellowship 1990.

Commonwealth Postgraduate PhD Scholarship 1981-1983.

PATENTS:

Patent - through the Council of the Queensland Institute of Medical Research and AMRAD Corporation Ltd. for the invention titled: "A novel growth factor and a genetic sequence encoding same".
Patent application number: PCT/AU96/00094.
* This patent covers the VEGFB gene and its therapeutic use.

Patent - through The Council of the Queensland Institute of Medical Research and AMRAD Corporation Ltd. for the invention titled: "A novel gene and uses therefor".
Patent application number: PCT/AU98/00380.
* This patent covers the RASGRP2 (MCG7) gene and its therapeutic use.

Provisional patent - through The Council of the Queensland Institute of Medical Research and AMRAD Corporation Ltd. for the invention titled: "A method of treatment and prophylaxis".
Patent application number PQ5921/00 (filed 29/02/00).
* This patent covers the therapeutic use of the VEGFB gene or related agonists or antagonists in treatment of rheumatoid arthritis.

SOCIETY MEMBERSHIPS:

Human Genetics Society of Australia
National Association of Research Fellows (NH&MRC)

COMMITTEE/PANEL MEMBERSHIPS:

Grant review panels

Cancer Research Grant Discipline Panel (Biochemistry, Molecular Biology and Genetics) – 2000

QCF Grant Review Panel 1991-current

Conference committees

Clinical Oncological Society of Australia 2001, Brisbane - Programme Committee 1999-current

Clinical Oncological Society of Australia 1996, Brisbane - Programme Committee 1995-1996

Australia and New Zealand Environmental Mutagens Society 1996, Mount Buller - Programme Committee 1995-1996

Queensland Cancer Fund International Conference on Melanoma 1994, Brisbane - Steering Committee 1992-1994

Institutional Committees

QIMR Comprehensive Cancer Research Centre Opening Scientific Conference 2001, Brisbane - Steering Committee 2000 - current

QIMR Scientific Advisory Committee 1999-current

QIMR Staff Association 1999-current

QIMR Comprehensive Cancer Research Centre User/Advisory Groups (Laboratory, Waste, Freezers) 1999-2000

QIMR Joint Consultative Committee, Faculty Representative 1998-current

QIMR Student Committee 1992-1994

QIMR Seminar Committee 1989

QIMR Library Committee 1986-2000

QIMR Conversazione Committee 1985-1992

EDITORIAL DUTIES:

Associate editor for *Clinical Cancer Research*

Editorial committee member for *Disease Markers*

JOURNAL REVIEWER FOR:

British Journal of Cancer
Cancer
Cancer Research
Clinical Cancer Research
Cell Stress and Chaperones
European Journal of Cancer
Genes Chromosomes and Cancer
Human Mutation
International Journal of Cancer
Journal of Gastroenterology and Hepatology
Journal of Investigative Dermatology
Journal of Medical Genetics
Oncogene

GRANT REVIEWER FOR:

Anticancer Council of Victoria
Australian Research Council
Clive and Vera Ramaciotti Foundations
Commonwealth Department of Veterans Affairs
Diabetes Australia Research Trust
Government Employees Medical Research Fund
Health Research Council of New Zealand
Leo and Jenny Foundation
National Health and Medical Research Council of Australia
National Institute of Forensic Science
New South Wales Cancer Council
Raine Foundation
United States-Israel Binational Science Foundation
University of Queensland Research Services Special Grants Scheme
University of Sydney Cancer Research Fund

INVITED CONFERENCE PRESENTATIONS:

5th World Conference on Melanoma – Venice – 1/3/01

Prevention of Melanoma and Skin Cancer Conference – Brisbane 5/12/00

Australian Society of Medical Research 39th Annual Meeting – Melbourne 28/11/00

Speaker and session chairman, American Association of Cancer Research – San Francisco 1/4/00

3rd Peter MacCallum Symposium – Initiation and Progression of Cancer – Melbourne 8/11/99

Combio Conference – Broadbeach 30/9/99

Lorne Cancer Conference - Lorne 1998

Royal Brisbane Hospital Health Care Symposium - Brisbane 1997

4th World Conference on Melanoma - Sydney 1997

Royal Australasian College of Surgeons Annual Scientific Congress - Brisbane 1997

The Mount Buller International Conferences on Environmental Radiation - The Environmental and Health Effects of Solar Radiation - Mount Buller 1996

23rd Clinical Oncological Society of Australia Conference - Brisbane 1996

7th Frank and Bobbie Fenner Conference in Medical Research - Molecular Mechanisms in Cancer- Canberra 1996

Australian Workshop on Cancer Gene Analysis and Mutation Detection - Noosa 1996

Italy-Australia Scientific Weeks 1996: Oncology - Brisbane 1996

10th Annual Oncology Nursing Symposium - Toowoomba 1994

Greenslopes Hospital Week Symposium - Brisbane 1993

Speaker and session chairman at the International Conference on Mutagens and Modulators of Gene Expression - Brisbane 1993

Lorne Cancer Conference - Lorne 1993

Speaker and session chairman at the Human Genetics Society of Australia meeting - Brisbane 1992

Melanoma Symposium - Brisbane 1988

Molecular Biology Symposium - Brisbane 1986

INVITED WORKSHOP PRESENTATIONS:

NCI Mouse Models of Human Cancer Consortium – Cutaneous Oncology – Puerto Rico 31/1/01

International Linkage Consortium for Familial Melanoma – Philadelphia 10/00

International Linkage Consortium for Familial Melanoma – Bethesda 6/00

International Linkage Consortium for Familial Melanoma - Bethesda 6/99

International Linkage Consortium for Familial Melanoma – San Francisco 10/99

International Linkage Consortium for Familial Melanoma - Bethesda 6/98

International Linkage Consortium for Familial Melanoma - Baltimore 10/97

Australian Cancer Genetics Consensus Meeting - Melbourne 1996

Session Chairman at the Fifth International Workshop on Multiple Endocrine Neoplasia - Stockholm 1994

American Society for Human Genetics 42nd Annual Meeting - Linkage analysis of familial melanoma - San Francisco 1992

International Association for the Study of the Liver Satellite Meeting - Molecular genetics of hepatocellular carcinoma - Brisbane 1990

OTHER CONFERENCE PRESENTATIONS:

In addition to the invited presentations listed above I also presented work at the following conferences:

1997	6th International Workshop on Multiple Endocrine Neoplasia, Noordwijkerhout (talk)
1995	45th American Society for Human Genetics Annual Meeting, Minneapolis (poster)
1994	44th American Society for Human Genetics Annual Meeting, Montreal (poster)
1990	41st American Society for Human Genetics Annual Meeting, Cincinnati (poster)
1990	12th Conference on Organization and Expression of the Genome, Lorne (poster)
1990	1st International Conference on DNA Fingerprinting, Berne (poster)
1990	International Association of the Study of the Liver Meeting, Broadbeach (poster)
1988	XII Human Genetics Society of Australasia meeting, Brisbane (poster)
1988	IV International Congress of Cell Biology, Montreal (poster)
1988	XVI International Congress of Genetics, Toronto (poster)
1987	31st Australian Biochemical Society meeting, Perth (talk plus poster)
1985	10th Australian Institute of Nuclear Science and Engineering Radiation Biology Conference, Sydney (talk)
1981	Australian Society for Medical Research, Brisbane (talk)

INVITED LECTURES AT TERTIARY INSTITUTIONS:

University of Queensland, Graduate Medical Course - 1997, 1998
Queensland University of Technology, Faculty of Life Sciences - 1991, 1992, 1993, 1995, 1996
University of Queensland, Biochemistry Department - 1984

INVITED SEMINARS:

National Institute for the Study and Cure of Cancers – Milan, Italy – 5/7/99
Children's Medical Research Institute, Sydney - 1997
Mater Misericordiae Hospital, Brisbane - 1997
Oncology Research Centre, Prince of Wales Hospital, Sydney - 1997
Wesley Hospital, Brisbane - 1996
Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, USA - 1995
AMRAD Corporation, Melbourne - 1995
Queensland Cancer Fund Oncology Nurses Group, Bundaberg - 1994
Centre for Molecular and Cellular Biology, University of Queensland - 1994
Mount Vernon Hospital, England - 1994
Ludwig Institute, Melbourne - 1993
Garvan Institute, Sydney - 1992
Westmead Hospital, Sydney - 1992
Biochemistry Department, University of Queensland - 1984
Royal Brisbane Hospital - 1980

STUDENTS:

I was the principal supervisor of the following graduates:

Sean Grimmond - PhD (University of Queensland) - 27/7/94 (subsequently obtained a C.J. Martin fellowship from the NHMRC)

Derek Kennedy - MMedSc (University of Queensland) - 17/2/95 (subsequently obtained a PhD from the University of Queensland)

Graeme Walker - PhD (Griffith University) - 23/2/95 (subsequently obtained a C.J. Martin fellowship from the NHMRC)

Lee Bergman - BSc(hons) (University of Queensland) - 11/97 - pass level = first class.

Daphne Macaranas - BSc(hons) (University of Queensland) - 11/97 - pass level = class 2A.

Derek Nancarrow - PhD (University of Queensland) – 30/7/99.

Pamela Pollock - PhD (University of Queensland) – 6/3/00 (subsequently obtained a C.J. Martin fellowship from the NHMRC)

I was a cosupervisor of the following graduates:

Teh Bin Tean - BMedSc (University of Tasmania) – 11/89 - (subsequently obtained a PhD from the Karolinska Institute, Stockholm, Sweden)

Hii Su Ing - BSc(hons) (Griffith University) – 11/93 - pass level = class 2A

Amanda Milligan - BSc(hons) (University of Queensland) - 11/95 - pass level = first class

Colin De Souza - PhD (University of Queensland) - 20/1/97

Ian Tonks - PhD (University of Queensland) - 24/3/97

Louise Sparrow - PhD (University of Western Australia) - 1998

Mark Romero – BSc (hons) (Griffith University) – 18/12/98 - pass level = first class

I am the principal supervisor of the following students:

Clare Boothroyd - MMedSc (University of Queensland) - submitted.

Christine Stewart - PhD (Queensland University of Technology) - currently in fourth year.

Lee Bergman - PhD (University of Queensland) - currently in third year.

I am a cosupervisor of the following students;

Jennifer Taylor – PhD (University of Queensland) - currently in third year.

Ellen Zevering - PhD (University of Queensland) - currently writing up/deferred.

Andrew Burgess - PhD (University of Queensland) - currently in first year.

In addition, I was a project supervisor for BC351 - University of Queensland Third Year Medical Molecular Genetics Course, and am a mentor for Literature Review topics within the Graduate Medical Course of the University of Queensland.

THESIS EXAMINER FOR:

Meryta May - BMedSc (University of Queensland) - 1993

Corinne Gustafson - PhD (University of Queensland) - 1996

David Walker - PhD (University of Queensland) – 1997/1998

MAJOR RESEARCH INTERESTS:

- Molecular and cellular biology of multiple endocrine neoplasia type 1.
- Linkage analysis, positional cloning and candidate gene analysis to determine novel familial melanoma genes (Collaboration with J. Trent and M. Brownstein).
- Use of cDNA expression microarrays to study cancer (Collaboration with J. Trent and S. Grimmond).

- Analysis of germline and somatic mutations in melanoma patients.
- Linkage and candidate gene analysis to determine the chromosomal position of genes involved in schizophrenia (Collaboration with B. Mowry and D. Nancarrow).
- Linkage and candidate gene analysis to determine the chromosomal position of genes involved in the development of moles (Collaboration with N. Martin).
- Generation of murine models of melanoma (Collaboration with G. Kay and G. Walker).
- Generation of murine models of multiple endocrine neoplasia type 1 (Collaboration with G. Kay).
- Study of the role of *Vegfb* in mouse development, pathology and neoplasia (Collaboration with G. Kay and A. Mould).

MAJOR SCIENTIFIC ACHIEVEMENTS:

- cloned and characterized the vascular endothelial growth factor B gene (*VEGFB*).
- cloned and characterized the *RASGRP2* gene – encoding a novel guanine nucleotide exchange factor.
- contributed to the independent isolation and characterization of the multiple endocrine neoplasia type 1 (*MEN1*) gene.
- contributed to the identification of the *CDK4* gene as a melanoma susceptibility locus.
- contributed to the generation of the first knockout mouse from QIMR (*Vegfb*).
- contributed to the generation of the first transgenic mouse from QIMR (antisense *Sod2*).
- first and only one to have carried out a linkage search covering the whole genome to determine the location of melanoma susceptibility genes.
- first to confirm the location of a melanoma susceptibility gene (*CDKN2A*) on chromosome 9.
- first to identify mutations of the *CDKN2A* gene in Australian melanoma families.
- first to identify mutations of the *MEN1* gene in Australian the multiple endocrine neoplasia type 1 families, and in sporadic endocrine tumours.
- first to publish linkage analysis of Australian breast cancer families.
- first to show that tumours from patients with the Beckwith-Wiedemann syndrome showed loss of somatic heterozygosity for markers on the short arm of chromosome 11, thus implicating this region as the location of a suppressor gene specific for these tumour types.
- first to show that pancreatic tumours from patients with MEN 1 showed loss of somatic heterozygosity for markers on the long arm of chromosome 11 around the *MEN1* gene.

- first in Queensland to develop human DNA fingerprinting.

FINANCIAL SUPPORT FOR RESEARCH:

CURRENT:

UQ-CRF 2000 $20000
Linkage disequilibrium mapping of a gene for mole development on chromosome 9p.
N. HAYWARD, N. MARTIN

QCF 2000 $52840
Epidemiological determinants of abnormal gene expression in cutaneous melanoma.
D. WHITEMAN, A. GREEN, N. HAYWARD

QCF 1999 $49512
2000 $50060
Development of mouse models of multiple endocrine neoplasia type 1.
N. HAYWARD, G. KAY, M. CUMMINGS.

NHMRC 1998 $133863 (NB: budget subsumed into institute block grant)
1999 $133863
2000 $133863
2001 $133863
Localization and cloning of genes for melanoma.
N. HAYWARD.

NHMRC 1997 $111170
1998 $112780 (NB: budget subsumed into institute block grant)
1999 $114388
Cloning and characterising the multiple endocrine neoplasia type 1 gene.
N. HAYWARD.

NHMRC 1996 $89388
1997 $93860
1998 $93860 (NB: budget subsumed into institute block grant)
Inheritance of susceptibility to melanoma.
J. AITKEN, N. MARTIN, N. HAYWARD, A. GREEN.

AMRAD 1/7/99 – 30/6/00 $137263
Characterization of *Vegfb* function *in vivo*
D. BELLOMO, A. MOULD, M. CAHILL, M. GARTSIDE, G. KAY, N. HAYWARD

PAST:

QCF 1998 $56514
1999 $56514
In vivo analysis of Vegf-B function.
G. KAY, M. CUMMINGS, N. HAYWARD.

AMRAD 1/7/98 – 30/6/99 $133129
Characterization of *Vegfb* function *in vivo*
D. BELLOMO, M. GARTSIDE, G. KAY, N. HAYWARD.

QCF 1996 $52331
1997 $53950
1998 $53950
Analysis of p16 in control of cell cycle and melanoma tumorigenesis.
B. GABRIELLI, M. CASTELLANO, N. HAYWARD.

AMRAD 1998 $25000
Molecular and functional characterisation of VRF/VEGF-B.
N. HAYWARD, G. SILINS, S. GRIMMOND.

NHMRC 1994 $106169
1995 $107867
1996 $112457
1997 $116796
Localization and cloning of genes for familial melanoma.
N. HAYWARD.

ARC 1997 $24000
Use of transgenic and knockout mice to study the physiological role of a novel growth factor.
G. KAY, N. HAYWARD

AMRAD 1997 $50000
Molecular and functional characterisation of VRF/VEGF-B.
N. HAYWARD, G. SILINS, S. GRIMMOND.

QCF 1996 $78921
Cloning the gene for multiple endocrine neoplasia type 1.
S. GRIMMOND, N. HAYWARD.

NHMRC 1994 $106169
1995 $107867
1996 $109593
Localization and cloning of genes for familial melanoma.
N. HAYWARD.

NHMRC 1994 $140081
1995 $124916
1996 $124916
A molecular genetic linkage study of schizophrenia.
B. MOWRY, N. HAYWARD.

QCF 1994 $50040
1995 $51040
1996 $51040
Development of a superoxide dismutase 2 transgenic model for melanoma.
K. ELLEM, G. KAY, P. PARSONS, N. HAYWARD

QLD UNI 1995 $20000
Role of the p16 gene in the development of melanoma.
N. HAYWARD.

AMRAD 1995 $49949
Characterisation of genomic structure and biological activity of a novel growth factor.
N. HAYWARD, S. GRIMMOND.

NHMRC 1993 $79061
1994 $79931
1995 $81210
Cloning of the multiple endocrine neoplasia type 1 susceptibility gene.
N. HAYWARD.

QCF 1994 $57505
1995 $58660
Genetic predictive testing of breast cancer susceptibility.
N. HAYWARD, N. WETZIG, I. BENNETT, B. TEH.

PAH 1993 $15000
1994 $15000
Genetic studies of breast cancer.
N. WETZIG, I. BENNETT, B. TEH, N. HAYWARD.

QCF 1991 $37455
1992 $41842
1993 $42680
Linkage analysis of chromosome 6 markers in familial melanoma.
N. HAYWARD.

QCF 1991 $46000
1992 $46000
Characterization of a centrosomal protein with an hTGFá related epitope.
K. ELLEM, N. HAYWARD

NHMRC 1990 $103930
1991 $65316
1992 $72609
Chromosomal mapping of genes involved in familial and sporadic melanoma.
N. HAYWARD, K. ELLEM.

QCF 1990 $37097
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N. HAYWARD, K. ELLEM.

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COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

OPPOSITION THERETO BY: Ludwig Institute for Cancer Research Under Section 59 of the Patents Act.

This is **Annexure NKH-2** referred to in my Statutory Declaration made this 8th day of December 2000.

N. Hayward

Nicholas Kim Hayward

WITNESS: _____

Commissioner for Declarations/Solicitor
Patent Attorney/Justice of the Peace

COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

DOCUMENT LIST

Documents provided to me by the Patent Attorneys representing HGS in the subject proceedings are as follows:

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